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This project was an attempt to generate a cultured neuron probe, in the form of a thin implantable silicon structure containing wells in which dissociated neurons were trapped before insertion. The sixteen wells each were to have an electrode for two-way extracellular communication with each neuron. It was hoped that probe neurons would integrate synaptically with the host, particularly if the probe neurons were of the same type as those in the region reached by the probe. The test system chosen was embryonic hippocampal pyrmidal neurons implanted in rat hippocampus.

The project had an initial phase of *in vitro* studies to develop and test silicon structures in easily controlled conditions. One study was to load a probe with hippocampal neurons and to then place it over a cultured hippocampal slice. This was successfully done, provided the probe was immersed in culture medium throughout the process. Outgrowth of cultured neurons ino the slice was observed to a depth of 100 microns, using a two photon laser-scanning microscope to record the outgrowth of live neurons with time-lapse imaging.

The other *in vitro* study was the creation of "neurochips" which contained a 4 x 4 array of wells on a flat silicon substrate. These wells firstly permitted studies of neural process outgrowth and containment in the wells. It was found that an initial design which worked for sympathetic neurons did not hold hippocampal cells successfully. The hippocampal cells had a strong tendency to escape, even through very small holes. After four iterations, a well design in which outgrowth took place through narrow tunnels was successful.

The neurochips were also used for studies of electrical stimulation and recording. Recording of action potentials from spontaneous and driven activity with excellent signal to noise ratio was achieved. For stimulation tests, the electrical recording of action potentials from the same cell that was stimulated was achieved, but with difficulty, since the stimulus artifact is more than 1000 times the recorded sugnal. More cleanly, voltage-sensitive dye recording was used to show that stimulation was uniformly successful, provided the electrodes were very well platinized. A technique for super-platinization was developed which guaranteed this.

In vivo studies were begun with "dummy probes", with wells that were not fitted with an electrode. These were loaded with septal neurons,

which provide cholinergic input to hippocampus, and were implanted in mature rats whose hippocampi had previously been denervated of all cholinergic inputs. With immunohistochemistry, outgrowth from a very small fraction of such neurons was observed after implantation for about one month. Further studies at Rutgers to achieve outgrowth of a large fraction of probe neurons were unsuccessful. It was found that almost all neurons were killed even by a very short term probe insertion and removal.

As the contract came to an end it was decided to move this phase of the work from Rutgers to Caltech, where Jim Bower's lab is supporting a continuing effort after the end of the contract. Very recently, success has been achieved in short term insertions and removal, with culturing afterwards to demonstrate excellent neural survival. The Pine lab has developed techniques for staining pyramidal cells with DiI so their outgrowth can be observed with fluorescence before and after insertion for these experiments. In addition, techniques for loading probes have been developed which produce high survival rates of controls that are not implanted. Finally, the insertion procedure has been modified to mimic that used successfully with slices, in which the probes are kept continually immersed.

The acute insertion experiments have progressed from five minutes duration to one hour. Plans are now being made to do long-term experiments, up to the time when DiI can still be seen in the two-photon microscope. This is up to about one month. A thick section containing the probe region can be observed with high resolution using this instrument, and outgrowth of the probe neurons into the surrounding tissue will be observable.

If the next phase of implantation experiments is successful with dummy probes, producing visible outgrowth into the rat hippocampus, then the next step for a new contract would be to proceed along the path outlined in the previous proposal: To demonstrate physiological integration of the probe with the host by observing the effects of probe stimulation and by using the probe to record stimulated activity generated in the hippocampus.